EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

REVISED & UPDATED

Edvo-Kit # 119

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Genes in a Tube™

Experiment Objective:

The purpose of the experiment is to rapidly extract "self" DNA from cheek cells, to visualize DNA, and to store DNA in a Genes in a Tube™ necklace.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

Component *Store entire experiment at room temperature.*	Check (✓)
• Lysis Buffer	❑
• Protease	❑
• Tris Buffer	❑
• Flash Blue™ solution	❑
• Salt packets	❑

Supplies	Check (✓)
• Clear tubes for DNA isolation	❑
• Microcentrifuge tubes with caps	❑
• Small transfer pipets	❑
• Calibrated transfer pipets	❑
• String for Genes in a Tube™ necklaces	❑
• Disposable plastic cup	❑

This experiment is designed for 26 DNA isolations.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Requirements *(not provided in the experiment)*

- Freezer/ice cold 95% Ethanol or isopropyl alcohol (rubbing alcohol)
- Water bath
- Test tube racks
- Ice and ice buckets
- Disposable laboratory gloves
- Centrifuge

Background Information

All living organisms are composed of cells. Organisms can either be single-celled, such as bacteria, or composed of many different cells. Complex organisms like humans are made up of billions of different cells. Both eukaryotic and prokaryotic cells contain a "genetic code", or a blueprint for how the cell functions. This genetic code is passed on from generation to generation when the cell or organism replicates. Eukaryotic cells contain organelles, which are specialized, individually enclosed structures within a cell with a specific function. For example, mitochondria are responsible for energy production and lysosomes are responsible for degradation of trash. In 1868 a biologist named Friedrich Miescher showed that the nucleus, a major organelle of eukaryotic cells, contained a material he named nucleic acid. While this was an interesting finding, it was almost 100 years later that this material in the nucleus was recognized as the carrier of the genetic code.

It is now well known that the genetic code is carried in the form of deoxyribonucleic acid (DNA). DNA is made up of deoxyribose carbohydrate units and nitrogenous bases. The structure of DNA was determined by James Watson, Francis Crick, and Rosalind Franklin in 1953. They determined that DNA was a double helix structure, meaning it looks like a twisted ladder. The sides of this ladder are made up of deoxyribose carbohydrates that are linked together by very strong covalent bonds. These sides are commonly referred to as the sugar-phosphate backbone and provide support for the rungs. The rungs of the ladder are actually a pair of nitrogen bases held together by hydrogen bonds, with one base on either side (Figure 1). The nitrogenous bases are adenine (A), cytosine (C), guanine (G), and thymine (T). Due to their structure, each base can only connect to one other base. The base pairings of the nitrogenous bases are A-T and G-C. So, if there is a single strand of DNA in the order of A-C-T-G, the opposite strand will be T-G-A-C.

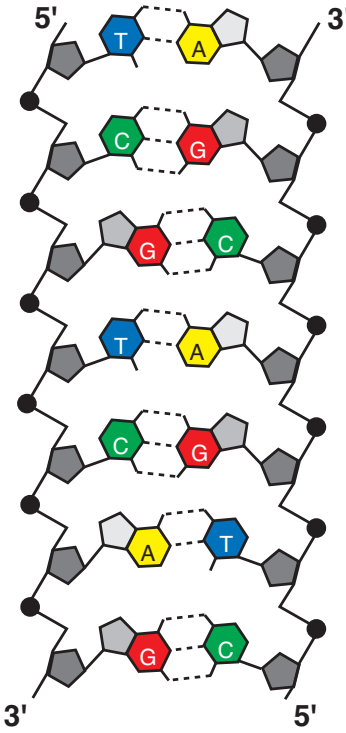


Figure 1

During the process of cell division, DNA provides the required information to copy (replicate) itself, which results in genetic information being passed on to the next generation of cells. DNA also provides the instructions for making proteins for various cell functions. Some of these proteins and enzymes are involved in the synthesis of DNA itself, such as the protein DNA polymerase. In addition to synthesis, DNA polymerases have the ability to edit newly synthesized DNA for possible errors in base pair matching and to help repair DNA that gets damaged during the life of the cell. Such damage to DNA can occur due to exposure to carcinogens and environmental factors such as X-rays and UV light from the sun.

The human genome consists of 2.9 billion base pairs. Of this total, only about 5% code for protein, such as those mentioned above. The remaining 95% of DNA are called noncoding sequences. Controversially, noncoding DNA used to be referred to as "junk DNA". However, today scientists are understanding that even if the DNA does not code for a specific protein, it is still important for biological function. For example, noncoding DNA can regulate protein creating by functioning as a specialized landing strip for enzymes like DNA polymerase; noncoding DNA can also hold sister chromatids together by helping to form a centromere.

DNA is located inside the nucleus of cells. In order to access it the cells need to be broken open, or lysed. DNA from chromosomes can then be released, isolated, and purified. DNA extraction is frequently the first step for molecular biology and biotechnology experiments. Extracted DNA is soluble in water and thus will appear as a clear solution. By contrast, DNA is insoluble in salt solutions and alcohol where it will precipitate to form white fibers.

Purification procedures for DNA usually include precipitation with alcohol in the presence of salt. The DNA solution is carefully overlaid with alcohol. Since alcohols such as rubbing alcohol (isopropyl alcohol) have a lower density than water, a second layer above the DNA solution will be formed. A glass rod or stirrer can be used to spool DNA at the interface of the two liquid phases and separate DNA from the solution (Figure 2). The DNA will appear as a viscous, clotted mass which can be collected on a stirrer or glass rod. The amount of DNA spooled will vary and is a consequence of the intactness of the DNA sample.

Figure 2

Almost any tissue or body fluid (except urine) may be used as a source of DNA. The most common sources of human DNA are samples from hair, cheek cells, blood, and saliva. Once extracted, DNA can be stored for long periods of time. Various methods of storage include precipitation and storage under alcohol at room temperature or refrigeration. DNA is often extracted for medical procedures medical procedures or as evidence left behind at crime scenes such as cells that are recovered from the fingernails of a victim. Even a few cells deposited by a person while licking and sealing an envelope can be sufficient to obtain DNA and identify the criminal.

In this experiment, individual "self" DNA will be extracted from cheek cells and stored in a "Genes in a Tube" necklace. DNA isolated from this experiment can be recovered and used for DNA fingerprinting and analysis of an individual's genes.

Experiment Overview

EXPERIMENT OBJECTIVE:

The purpose of the experiment is to rapidly extract "self" DNA from cheek cells, to visualize DNA, and to store DNA in a “Genes in a tube™” necklace.

LABORATORY SAFETY:

Wear gloves and safety goggles

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.
- Contaminated laboratory waste (saliva solution, cup, pipette, etc.) must be disinfected with 15% bleach solution prior to disposal. Be sure to properly dispose any biological samples according to your institutional guidelines.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

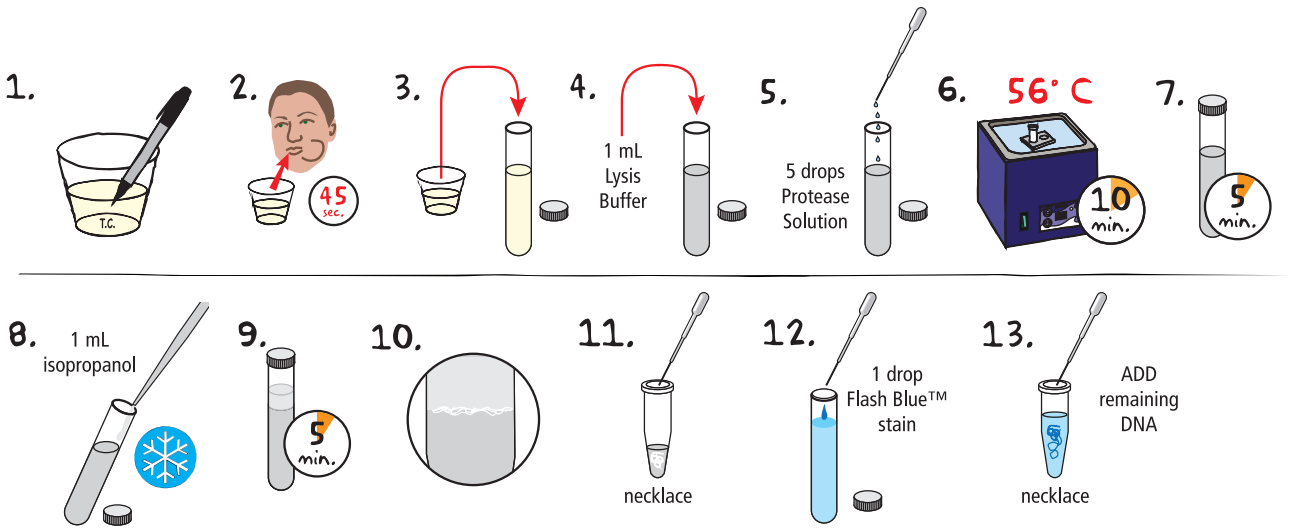
During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

DNA Isolation from Cheek Cells



1. **OBTAIN** a cup containing 1 mL saline solution from your instructor. Clearly label the cup with your initials or name.
2. **RINSE** your mouth vigorously for 45 seconds. Gently chew on the inside of your cheek to release more cells (no blood!!). **EXPEL** the solution back into the same cup.
3. Using large transfer pipet, **TRANSFER** the entire saline solution to a clean capped tube.
4. Using large transfer pipet, **ADD** 1 mL Lysis Buffer to the tube.
5. Using a small transfer pipet, **ADD** 5 drops Protease Solution to the tube. Pipet up and down to **MIX**.
6. **INCUBATE** the tube for 10 minutes at 56° C.
7. **REMOVE** the tube from the water bath or incubator and let **REST** at room temperature for 5 minutes.
8. Using a transfer pipet, hold the test tube at a 45 degree angle and gently **STREAM** 1 mL of ice cold isopropanol down the side of the tube.
9. **PLACE** tube in a test tube rack sitting upright. Let the tube **REST** for 5 minutes at room temperature.
10. **CHECK** that your DNA is visible at the interface of alcohol/lysis solution. Some DNA may appear as stringy material, grayish-white in color. It may even have bubbles. This material is your DNA!
11. Using a small transfer pipet, **TRANSFER** a small amount of DNA to the Genes in a Tube™ necklace.
12. **STAIN** the remaining DNA by using a small transfer pipet to **ADD** 1 small drop of the Flash Blue™ to the test tube.
13. **TRANSFER** your remaining DNA to the Genes in a Tube™ necklace.

NOTE:
Flash Blue™ is a DNA stain which will make the extracted DNA more easily visible to the naked eye. Your DNA will appear as heavy blue gel-like strands at the alcohol and aqueous interface. The solution will also have a light blue color but will be lighter than your DNA.

Study Questions

1. Describe the appearance of the isolated DNA.
2. What do cell nuclei contain?
3. What does cell lysis mean?
4. Why did the rubbing alcohol form a layer on top of the DNA solution?

Instructor's Guide

NOTES TO THE INSTRUCTOR:

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in planning and implementing this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Preparation For:	What to do:	When:	Time Required:
Isolation of DNA from Cheek Cells	Prepare and aliquot various reagents (saline).	Up to one day before performing the experiment.	30 min.
	Prepare and aliquot Lysis Buffer.	Prepare on the day the students will be performing the experiment **OR** freeze for up to one week.	15 min.
	Equilibrate water bath at 56° C	Anytime before performing the experiment.	5 min.

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

Pre-Lab Preparations

1. Add 200 µL of Tris buffer to the tube of protease. Allow the material to hydrate for a few minutes and transfer the entire amount back to the remaining Tris buffer.

   Mix well and aliquot 100 µL for each student pair into microcentrifuge tubes. Be sure to place the tubes on ice until they are needed.

2. Aliquot 3 mL of Ethanol or Isopropyl alcohol for each student pair into microcentrifuge tubes. Place on ice until needed.

3. Aliquot 200 µL of Flash Blue solution for each student pair into microcentrifuge tubes.

4. Aliquot 1 mL of Lysis Buffer into 2 mL microcentrifuge tubes - one per student.

5. Dissolve all 8 salt packets in 500 mL of drinking water to make the saline solution. Aliquot 1 mL per cup per student. ***NOTE: do not aliquot more than 1 mL because the resulting solution may not fit into the test tube after lysis buffer and alcohol are added.***

For this experiment, each student needs:
- 1 mL lysis buffer
- 1 clear tube with cap
- 3 small transfer pipets
- 3 large transfer pipets
- 1 tube & string to make the Genes in a Tube™ necklace
- 1 cup of saline solution (1 mL)

Reagents to be shared by two students:
- 100 µL Protease solution
- 200 µL Flash Blue™ solution
- 3 mL Ice cold alcohol (95% ethanol or Isopropyl rubbing alcohol)

Inquiry-based Optional Extension Experiments

Encourage students to think of different parameters in the experiment to alter and have them predict the outcome. Listed below are several examples of optional activities for students to try.

- Perform an experimental control with just the lysis buffer (cheek cells are not added). Add the protease and overlay the solution with alcohol. Note the differences between this control tube and the sample tubes containing DNA.
- Perform the DNA isolation and eliminate the protease and/or alcohol from the isolation steps. What happens to the DNA sample?
- After the addition of alcohol, isolate the DNA by spooling. DNA can be recovered by dissolving in distilled water overnight and then prepared for agarose gel electrophoresis (materials for electrophoresis not included).
- Isolate the DNA and perform PCR on the DNA sample (materials for PCR not included).

Expected Results

Genes in a Tube™

Please refer to the kit insert for the Answers to Study Questions